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(54) Title: CUTINASE CLEANING COMPOSITION

(57) Abstract

This invention relates to cleaning compositions and methods for using them. Particularly, the invention relates to compositions comprising a surfactant and a cutinase enzyme.

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Cutinase Cleaning Compositions

Background of the Invention

a) Field of the Invention

The invention relates to enzymatic cleaning compositions and methods for using them. Particularly the invention relates to cleaning compositions comprising a surfactant and a cutinase enzyme.

b) Background Information

A wide variety of enzymes are well known for use in cleaning compositions. The use of B. subtilisins and B. licheniformis protease in commercial preparations is common. Other enzymes have also been used in commercial cleaning compositions such as, for example, U.S. Patent No. 4,011,169, and British Patnet No. 1,293,613. Also a comprehensive review article of lipases in cleaning compositions can be found in Journal of Applied Biochemistry, 2:218-229 (1980) in an article entitled "Lipases as Detergent Components". Lipolytic detergent additives are also known from e.g., British Patent Specification No. 1,293,613 and Canadian Patent No. 835,343.

U.S. Patent No. 3,950,277 and British Patent Specification No. 1,442,418 disclose lipase enzymes combined with an activator and calcium and/or magnesium ions, respectively, which are utilized to pre-soak soiled fabrics and to remove triglyceride stains and soils from polyester or polyester/cotton fabric blends, respectively, Suitable microbial lipases for use therein (apart from animal and plant derived lipases) are said to be those derived from Pseudomonas, Aspergillus, Pneumococcus, Staphylococcus, and Staphylococcus toxins, Mycobacterium tuberculosis, Mycotorula lipolytica, and Sclerotinia.

British Patent Specification No. 1,372,034 discloses a detergent composition comprising a bacterial lipase produced by Pseudomonas stutzeri strain ATCC 19154. Furthermore, it is recommended that the preferred lipolytic enzymes should have a pH optimum between 6 and 10, and should be active in said range, preferably between 7 and 9. Around 1970, this presumed Pseudomonas stutzeri strain was reclassified as Pseudomonas aeruginosa, as appears for example from the ATCC catalogues.

European Patent Application EP-A-0130064 discloses an enzymatic detergent additive comprising a lipase isolated from Fusarium oxysporum with an alleged higher lipolytic cleaning efficiency than conventional lipases.

In European Patent Application No. 0214761, Enzymatic detergent additives are described as the active component of which is a microbially produced lipase from a strain of Pseudomonas cepacia. The lipases described therein are claimed to be superior to the lipolytic detergent action of the prior art, especially at low temperature washing processes (around 60°C and below).

In PCT Patent Application No. 87/00859 other novel lipolytic enzymes are described as having an optimal pH in the range of 8 - 10.5 at a temperature of 60°C or less from bacterial strains selected from Pseudomonas pseudoalcaligenes, P. stutzeri and Acinetobacter calcoaceticus. These enzymes are described as particularly effective at low temperatures; i.e., 40°C or lower and effective in both liquid and solid detergent compositions.

Also in U.S. Patent No. 3,950,277, it is described in general terms that lipases from Pseudomonas are suited as agents for removal of oily stains from fabrics, if used together with a special group of lipase activators. The art cited does not, however, cover cutinase enzymes from Pseudomonas or any other microbial source. However, prior art enzymes for use in cleaning compositions, while effective on many proteins and lipids, are not completely effective against all stains commonly found in laundry and other cleaning applications.

Further, many lipases are not stable at pH 8 - 11 where most cleaning compositions are used. Even further, most enzymes for use in cleaning compositions are not very stable, if at all, under oxidative conditions or in the presence of other enzymes such as proteases.

SUMMARY OF THE INVENTION

Accordingly it has been discovered that combinations of a surfactant and a substantially pure microbial cutinase enzyme are effective compositions for cleaning applications. The cutinase enzyme preparations possess activity at pH of from about 8 and 11, exhibit cleaning activity in aqueous solution at concentrations from about .05 mg/L to about 100 mg/L or more at temperatures from about 20°C to about 50°.
The enzymes are oxidatively stable and stable in the presence of other enzymes such as proteases. Even further, the cutinases show a synergistic effect when a plurality of surfactants are used with the cutinase.

The invention also relates to the improved process for enzymatically cleaning a material with an aqueous solution; the improvement comprising adding a substantially pure cutinase to the cleaning solution.

DETAILED DESCRIPTION OF THE INVENTION

Applicant has discovered that cutinase enzymes are useful when included in cleaning compositions. These compositions may take on a variety of forms such as for laundry cleaning, household and industrial cleaning, and the like. The cleaning compositions comprise combinations of known surfactants and a microbial cutinase enzyme which can be used to clean a wide variety of materials. The composition can be added to aqueous solution or solid powder, or formulated in an aqueous solution or solid powder and used according to conventional cleaning techniques.

Enzyme

Cutinases are well known in the art and are available from a wide variety of sources. See Cutinases from Fungi and Pollen, P.E. Kolattukudy, pg. 472 - 504, incorporated herein by reference, for discussion of cutinases useful in the practice of the invention. A preferred cutinase is that cutinase isolated in a substantially pure form from Pseudomonas putida, particularly the P. putida, ATCC 53552, described in copending U.S. patent application, Serial No. 932,959 filed November 19, 1986 and incorporated herein by reference, which enzyme therefrom has the following amino acid sequence:

1									10		
ala	pro	leu	pro	asp	thr	pro	gly	ala	pro	phe	pro
							20				
ala	val	ala	asn	phe	asp	arg	ser	gly	pro	tyr	thr
					30						
thr	ser	ser	gln	ser	glu	gly	pro	ser	cys	arg	ile
			40								
tyr	arg	pro	arg	asp	leu	gly	gln	gly	gly	val	arg
	50									60	
his	pro	val	ile	leu	trp	gly	asn	gly	thr	gly	ala
									70		
gly	pro	ser	thr	tyr	ala	gly	leu	leu	ser	his	trp
							80				
ala	ser	his	gly	phe	val	val	ala	ala	ala	glu	thr
					90						
ser	asn	ala	gly	thr	gly	arg	glu	met	leu	ala	cys
			100								
leu	asp	tyr	leu	val	arg	glu	asn	asp	thr	pro	tyr
	110									120	
gly	thr	tyr	ser	gly	lys	leu	asn	thr	gly	arg	val
									130		
gly	thr	ser	gly	his	ser	gln	gly	gly	gly	gly	ser
							140				
ile	met	ala	gly	gln	asp	thr	arg	val	arg	thr	thr
					150						
ala	pro	ile	gln	pro	tyr	thr	leu	gly	leu	gly	his
			160								
asp	ser	ala	ser	gln	arg	arg	gln	gln	gly	pro	met
	170										180
phe	leu	met	ser	gly	gly	gly	asp	thr	ile	ala	phe
									190		
pro	tyr	leu	asn	ala	gln	pro	val	tyr	arg	arg	ala
							200				
asn	val	pro	val	phe	trp	gly	glu	arg	arg	tyr	val
					210						
ser	his	phe	glu	pro	val	gly	ser	gly	gly	ala	tyr
			220								
arg	gly	pro	ser	thr	ala	trp	phe	arg	phe	gln	leu
	230									240	
met	asp	asp	gln	asp	ala	arg	ala	thr	phe	tyr	gly
									250		
ala	gln	cys	ser	leu	cys	thr	ser	leu	leu	trp	
ser	val	gly	arg	arg	gly	leu					

Other sources of bacterial and fungal cutinases include:

Fusarium solani pisi
Fusarium roseum sambucinum
Fusarium roseum culmorum
Helminthosporum sativum
Ulocladium consortiale
Streptomyces scabies
Colletotrichum capsici
Phytopthora cactorum
Botrytis cineria
Colletotrichum gloeosporioides

The cutinase of the invention should preferably be selected to cause at least about 10%, and preferably 20%, hydrolysis of the given fat under given conditions. Normally the amount would be in a concentration of from about .01% to about 5.0% by weight of the surfactant, and preferably from about .05% to about 3%, such that upon dilution in wash water it is in a concentration of at least about .05mg/L. Further, one skilled in the art could take the preferred cutinase or, for that matter, any cutinase of the invention or any immuniologically identical cutinase and use random or selective replacement of amino acids to produce other cutinases which are more or less selective toward given substrates or include modification in activity such as oxidative stability. Substantially pure cutinase includes the isolated enzyme as well as the broth containing the enzyme in unpurified form but essentially free of other enzymes and enzyme sources.

The natural substrate of cutinase is cutin which is a biopolyester polymer which covers the plant leaves, fruits, etc., see Structure, Biosynthesis and Biodegradation of Cutin and Suberin. (1981), P.E. Kolattukudy, Ann. Rev. Plant Physiol., 32, pgs. 539-567. Stains comprising lipids which could be hydrolyzed or bound by cutinase on a substrate such as cloth would be similar to the natural substrate cutin. Cutinase, for these types of stains, will be more

effective than the prior art lipases. The cutinases will work especially well on gravy, oils and greases, plant or grass, oil based makeup and collar stains.

Cutinases are distinguishable from other lipases by methods well known in the art, See R.E. Purdy and P.E. Kolattukudy, Biochemistry, "Cutinase Assay", 14:2831-2840, (1975). Microbial cutinases from both fungal and bacterial sources have very good activity at pH 8 - pH 11 which is an ideal pH condition for detergent use.

Because of the specific activity of cutinases, it is a preferred aspect of the invention to combine one or more other cutinases, or one or more other enzymes, such as proteases, amylases or other lipases, along with the cutinase of the invention in the cleaning composition. Further, Applicant shows a synergistic increase in hydrolytic activity of cutinase when two or more surfactants are combined along with the cutinase enzyme.

Cutinases then are ideal for cleaning composition inclusion. They have stability oxidatively such as in H_2O_2. They have good stability in a temperature range of from about 20 - 50°C which is ideal from a cleaning point of view. They are also stable in the presence of other enzymes; e.g., proteases, and as such, are ideal for mixtures of enzymes.

The Surfactant

A number of known compounds are suitable surfactants useful in the present compositions. These include nonionic, anionic, cationic, or zwitterionic detergents, as disclosed in U.S. 4,404,128 to Barry J. Anderson and U.S. 4,261,868 to Jiri Hora et al. The art is familiar with the different formulations which can be used as cleaning compositions.

The Cleaning Compositions and Method of Use

Cutinases can be formulated as a purposefully added ingredient into known powdered and liquid detergents having pH between 6.5 and 12.0 at levels of about 0.01 to about 5% (preferably .05 to .5%) by weight of the detergent. These detergent cleaning compositions can also include other enzymes such as known proteases and amylases, as well as bleaches, colorants, builders, and stabilizers.

The cutinase of the invention may be added to powdered detergents in the form of granulates or prills, prepared by methods known in the art such as described in British Patent Nos. 1,324,116 and 1,362,365 and U.S. patent Nos. 3,519,570; 4,106,991 and 4,242,219.

The cutinase preparations of the invention can be prepared by cultivating the microorganisms defined herein or otherwise cutinase containing microorganism under appropriate conditions. In order to obtain reasonable yields of enzyme, media containing readily assimilable carbon and energy sources as necessary such as a nitrogen source, as well as calcium and magnesium salts and trace elements and cutin, or monomers of cutin, or compounds resembling cutin or cutin monomers. One could also obtain the gene for cutinan and express in any organism of choice where one may not have to add cutin or cutin monomers into the fermentation.

The addition of cutinase to conventional cleaning compositions does not create any special use limitation. In other words, any temperature and pH suitable for detergent compositions containing enzymes is also suitable for the present compositions.

Although the preferred form of the invention has been described above, it will be obvious to those skilled in the art to which the invention pertains, that, after understanding the invention and in view of the following testing as a whole, various changes and equivalent modifications may be made without parting from the scope of the invention as defined by the claims.

STABILITY CUTINASE AGAINST PROTEASES

Reaction conditions:

Buffer: 0.1 M NaP, pH 10

Temp: 37°C

Lipase: 42 ug/ml

Approximately 1:1 protease:cutinase aqueous solution were made up with the following results.

ENZYME ACTIVITY

Protease					
Incubation Time	0 min	5 min	10 min	15 min	14 hrs
None	1.57	1.60	1.47	1.63	1.61
Maxacal (35ug/ml)	1.68	1.58	1.72	1.66	0.134
Esperase (64 ug/ml)	1.73	1.64	1.59	1.51	0.456

Maxical is Gist-Brocade's brand of subtilisin enzyme (protease)

Esperase is Novo's brand of protease enzyme (protease)

TEMPERATURE STABILITY OF BACTERIAL CUTINASE

HALF LIFE AT 50°C

pH	Hrs.
7	30
8	25
9	12
10	0.3

Enzyme was incubated at 50°C in 0.1M sodium phosphate buffer at various pH's and activity was measured by hydrolysis of trioctanoin in polyvinyl alcohol emulsions.

EFFECT OF DETERGENTS ON HYDROLASE ACTIVITY

Reaction Conditions:

substrate: p-nitro-phenyl butyrate, 1mm (pnp)

pH: 8.0

buffer: 0.1m tris pH 8.0

temperature: 25°C

enzyme: bacterial cutinase from ATCC 53552

An aqueous solution with the following were made up and the enzyme activity was measured in these solutions using pnp as a substrate by following absorbance of p-nitrophenol at 410 mm.

Triton x100%	SDS %	% Activity
0	0	100
0.2	—	78
0.4	—	60
—	0.05	30
—	0.1	23
—	0.2	14
—	0.4	6
0.4	0.4	78
0.2	0.2	98
0.2	0.05	125
0.2	0.1	138

6.1	0.1	130
0.05	0.05	132

1) Non-ionic detergent inhibition is not significant at low concentrations.

2) An ionic detergent inhibitor at high concentrations.

3) Mixture of anionic and non-ionic detergents stimulate activity.

STABILITY TOWARD OXIDANTS

Cutinase .5 mg/ml in 0.1m sodium phosphate buffer, was incubated with various levels of hydrogen peroxide at pH 8.4 and 25°C for 2 hours, and hydrolytic activity was measured by a pH-stat using trioctanoim-polyvinyl alcohol emulsion.

[H_2O_2 ppm]	Hydrolytic Activity % Remaining
0	100
100	86
200	86
500	91
1000	95

TABLE 1

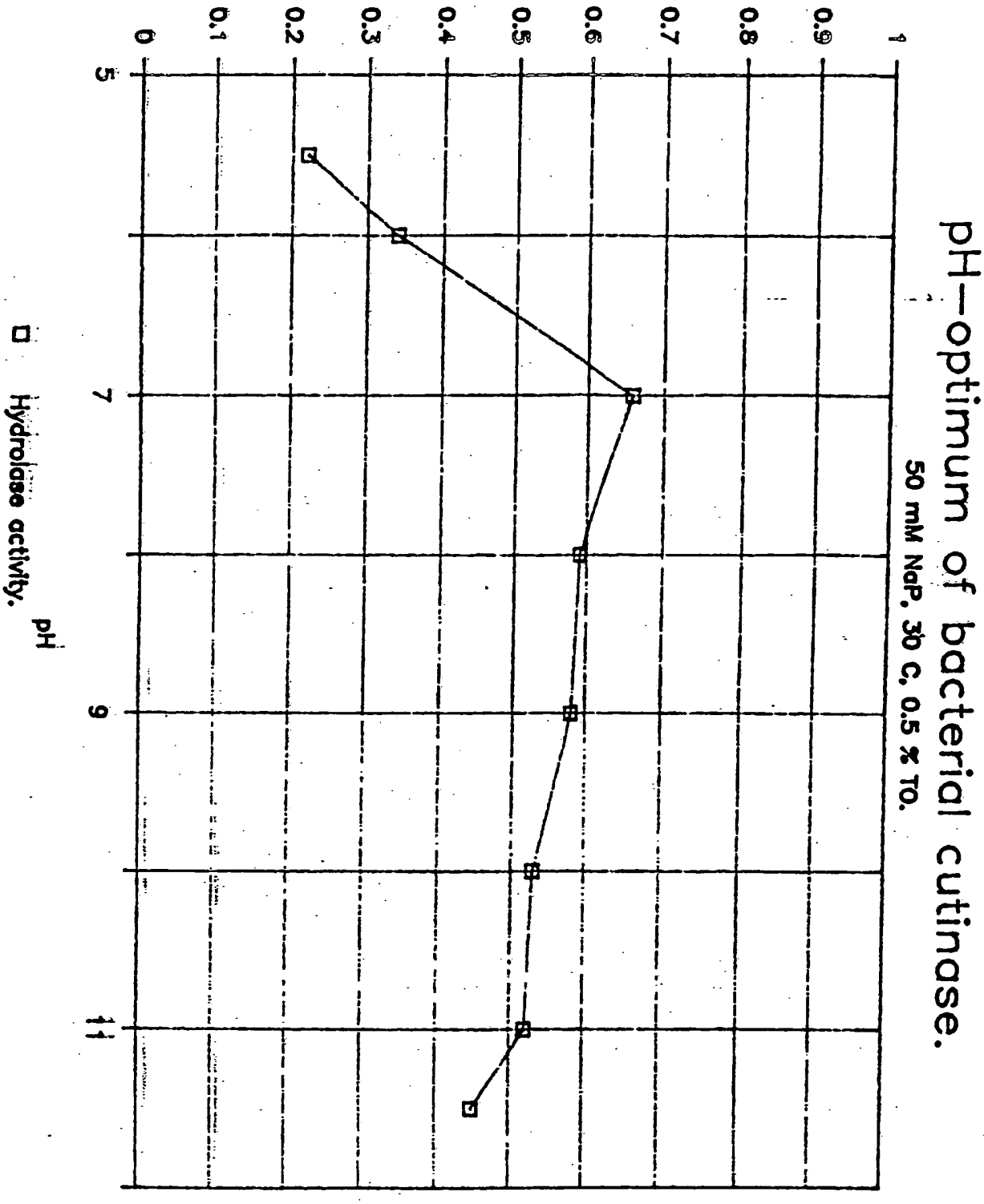
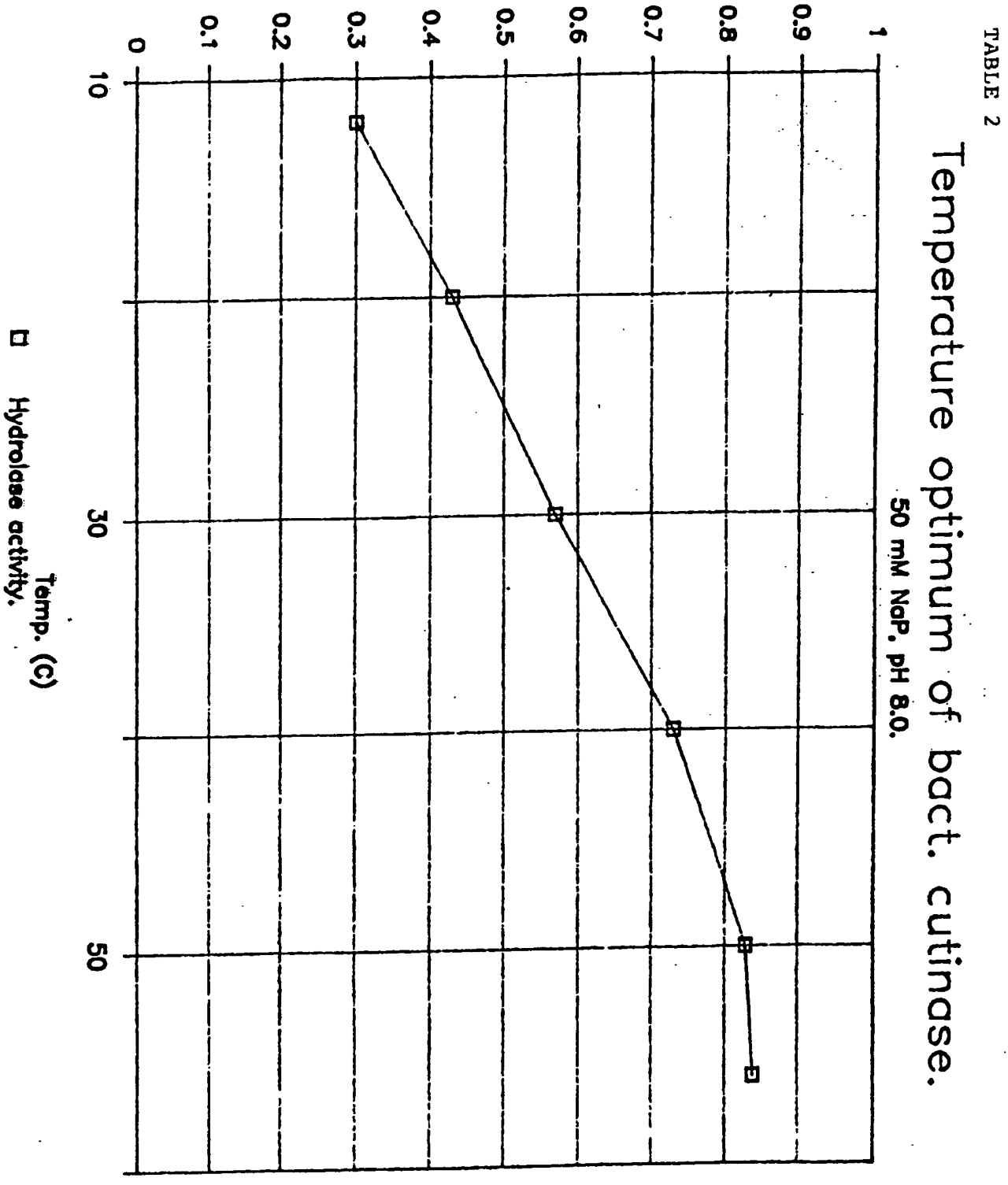

pH—optimum of bacterial cutinase.
50 mM NaP, 30 C, 0.5 % To.
Act. (mmol/mg.min)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
5
7
9
11
pH
Hydrolase activity.

TABLE 2


Temperature optimum of bact. cutinase.
50 mM NaP, pH 8.0.
Act. (mmol/mg.min)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
10
30
50
Temp. (C)
Hydrolase activity.

What is Claimed is:

1. An enzymatic cleaning composition comprising a substantially pure microbial cutinase in an amount sufficient that upon dilution in an aqueous solution for use there is sufficient cutinase to achieve a concentration of from about .05 mg/L to about 100 mg/L of cutinase.

2. A composition according to Claim 1 wherein the cutinase is the cutinase present in an amount of from about 0.01% to about 5% by weight of the surfactant.

3. A composition according to Claim 2 wherein the Pseudomonas putida is ATCC 53552.

4. A composition according to Claim 1 which further comprises one or more enzymes selected from lipases, amylases or proteases.

5. A composition according to Claim 1 wherein the cutinase selected is capable of causing at least 10% hydrolysis of a cutin or cutin-like substrate desirous of being cleaned from a given surface.

6. A composition according to Claim 1 wherein the cutinase is a non-naturally occurring cutinase having at least one amino acid randomly or selectively replaced by an amino acid not naturally found at that position.

7. A composition according to Claim 1 wherein the cutinase is added in the form of cell fermentation broth containing cutinase but essentially free of other enzymes or enzyme sources.

8. An enzymatic cleaning additive wherein the active component which is a substantially pure microbially produced cutinase.

9. A composition according to Claim 8 which further comprises one or more enzymes selected from lipases, amylases or proteases.

10. A composition according to Claim 8 comprising a plurality of surfactants.

11. A composition according to Claim 10 wherein the surfactants are SDS and Triton X-100.

12. An improved method for enzymatically cleaning a material having a cutin or cutin-like stain comprising:
a) selecting a cutinase enzyme;
b) forming an aqueous solution with said enzyme in a concentration of from about 0.05 mg/L to about 100 mg/L;
c) contacting the material with the solution of step b); and
d) rinsing the material of step c).

13. A method according to Claim 12 wherein the aqueous solution further comprises a surfactant compatible with the selected cutinase.

14. A method according to Claim 13 wherein the aqueous solution further comprises a surfactant compatible with the selected cutinase.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/01844

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 11 D 3/386

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 11 D; C 12 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
P,X	EP, A, 0268452 (GENENCOR) 25 May 1988 see example 13; abstract; page 1, lines 29-30 cited in the application	1-3,5,6,8, 12,13

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* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29th August 1988	20 SEP 1988
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8801844 SA 22728

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 08/09/88 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0268452	25-05-88	AU-A- 8115387	26-05-88
		AU-A- 7901387	09-06-88